

Changes of Mycetocyte Symbiosis in Response to Flying Behavior of Alatiform Aphid (*Acyrtosiphon pisum*)

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ABSTRACT—Prior to migratory flight of alatiform aphids, their total volume of mycetocyte was greatly reduced. Since in this period they ingest little food, it is likely that they develop the flight muscles at the cost of their mycetocytes. In this period, not only the size and number of the mycetocyte but also the density of endosymbionts in the cell decreased. Starving aphids resulted in a sharp decrease in the total volume of mycetocyte, which was reversed by refeeding, suggesting that aphids consume their mycetocytes harboring endosymbionts as a nutrient source on their physiological demands.

INTRODUCTION

Most groups of the family Aphidoidea harbor prokaryotic endosymbionts within specialized cells, mycetocytes, in the haemoceal [2, 5]. The symbiosis between aphid and its symbiont has so highly evolved that one can no longer do without the other. Aposymbiotic aphids produced by treating with antibiotics exhibit severe defects such as retarded growth and low fecundity, while the symbionts isolated from aphids cannot multiply themselves [6]. Although some indirect evidence suggests that the symbionts synthesize and supply vitamins, amino acids, and sterols to the host [2, 4, 5, 10, 11, 12], there still remain many questions unanswered about the relationship between aphid and its symbiont.

In order to further clarify this symbiotic interaction, it will be essential to know how the physiological conditions of aphid affect the mycetocyte in which the symbionts are housed. Douglas and Dixon reported variations in number and size of mycetocytes of *Megoura viciae* and *Acyrtosiphon pisum* with age and morph in detail [3]. They revealed that the number of mycetocytes decreases consistently after birth, and that the reported rate of decline is higher in alatae than in apterae. They also reported that the mycetocyte size increases over larval stages and soon after the final ecdysis the median size shows a declining curve in both apterae and alatae are due to the difference in growth rate, or number of embryos which vertically receive symbionts.

Unlike apterae, alatae are driven to flight a few days after the final ecdysis to settle on new host plants and start larviposition [8]. During this period alatae undergo morphological and physiological changes such as hardening of the wing and cuticle, and degeneration of the flight muscles.

We studied the changes of mycetocytes in the two morphs of *A. pisum* after the final ecdysis. The data showed that the marked changes in number, size, and color of

mycetocytes take place over the first several days. It was also revealed that in the same period the density of endosymbionts in the mycetocyte changes considerably. We also studied the effect of starvation on the condition of the mycetocyte of the two morphs. The present results will lend a clue to look into how the host controls the symbionts in response to its own physiological conditions.

MATERIALS AND METHODS

Insect materials

A long established parthenogenetic clone of pea aphids, *Acyrtosiphon pisum* (Harris) was maintained on young broad bean plants, *Vicia faba* (L.) at 20°C with photoperiod of 16 hr. Uniform nutritional conditions were retained in the following procedures. Adult apterae were placed on seedlings of the two-leaf stage to obtain nymphs with the average density of 10 per seedling. To obtain alatae, parental apterae were kept in a relatively high density.

Under these conditions used, the 4th stage spanned from day 5 to 7 of birth, the final ecdysis occurred on day 7 or 8, and the larviposition started on day 9 or 10 (apterae) and day 11–13 (alatae).

Starvation of insects

Starvation of insects was conducted on 1–2 days after the final ecdysis, when their cuticle had not completely hardened yet, indicating that the insects were before flight.

The aphids to be starved were transferred to petri dishes with moist filter papers to prevent desiccation, and returned to fresh host plants 3 days later.

Measurement of number and size of mycetocytes

The mycetocytes were isolated by dissecting insects submerged in buffer (35 mM Tris-HCl, pH 7.6 containing 10 mM MgCl₂, 25 mM KCl and 250 mM sucrose) on petri dish covered with agars, and the number and diameter of mycetocytes were determined under a stereoscopic microscope (15×6.3). In this method, mycetocytes, unless very small in size, were easily discriminated from other fat body cells and embryos. The number of mycetocytes was expressed as mean ± SD. The volume of mycetocytes was calculated from the diameter measured with a micrometer and expressed as mean ± SD of median values of about 30 round-shaped cells per individual. The total volume of mycetocytes was calculated based on the number and

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mean diameter for each individual and expressed as mean $\pm$ SD.

Relative growth rates of the aphids and their mycetocytes were calculated from the fresh body weight and volume, respectively [1].

The statistic analysis was conducted on Welch's method.

Density of endosymbionts

The density of endosymbionts in the mycetocyte was estimated measuring the number of symbiont in $0.5\text{ }\mu\text{m}$ -thick sections of mycetocytes under a light microscope. The isolated mycetocytes were fixed in the phosphate-buffered (pH 7.6) 1.5% formaldehyde - 1.5% glutaraldehyde fixative, then dehydrated, cleared, and embedded in resin (Technovit 7100, Kulzer). The sections were stained with Toluidine blue or hematoxylin-eosin.

RESULTS

Number and size of mycetocyte

The changes in number and size of the mycetocytes with age and morph are shown in Fig. 1.

Most alatae used in this study exhibited the flight behavior on day 2-4 of final ecdysis. They were usually found walking on the wall of the experimental cage, and returned to the host plants within a day. For convenience's sake, only the insects before flight were referred to day 1, those showing the flight behavior were referred to day 3, and those settling on host plants after flight were referred to day 4.

After the final ecdysis, the mean number of mycetocytes per aphid decreased slowly in apterae (-15% , $P < 0.01$,

between day 0 and day 3) and rapidly in alatae (32% , $P < 0.01$, between day 0 and day 3) (Fig. 1). In alatae, the decrease in number stopped on the re-settling on the host plant on day 4, and the number of mycetocytes somewhat increased thereafter ($P < 0.05$, between day 3 and day 9) (Fig. 1), though no dividing mycetocyte was detected throughout this period under a microscope.

The median size of mycetocyte was changed with age, also in a different manner between in apterae and in alatae (Fig. 2). In apterae, the size increased regularly over 4th instar stage to the beginning of larviposition ($P < 0.01$, between day 0 and day 3). The relative growth rates (r.g.r) from day -1 to 2 were $0.29\text{ mm}^3\text{ mm}^{-3}\text{ day}^{-1}$ and $0.30\text{ mgmg}^{-1}\text{ day}^{-1}$ for mycetocyte and insect, respectively. Afterward, the size of mycetocyte was kept unchanged or slightly decreased (Fig. 2). By contrast, in alatae, the size of mycetocyte decreased steeply between day 0 and day 3 ($P < 0.01$, r.g.r. being $-0.57\text{ mm}^3\text{ mm}^{-3}\text{ day}^{-1}$ and $-0.18\text{ mgmg}^{-1}\text{ day}^{-1}$ for mycetocyte and insect, respectively). Then the size turned to a sharp increase to restore that on day 0 ($P < 0.01$, between day 3 and day 4; $P < 0.01$, r.g.r.: $0.28\text{ mm}^3\text{ mm}^{-3}\text{ day}^{-1}$, $0.15\text{ mgmg}^{-1}\text{ day}^{-1}$, between day 3 and day 9,) (Fig. 2).

Figure 3 shows the change in total volume of mycetocytes per aphid. In apterae, the total volume increased until the onset of larviposition ($P < 0.01$, r.g.r.: $0.21\text{ mm}^3\text{ mm}^{-3}\text{ day}^{-1}$, $0.30\text{ mgmg}^{-1}\text{ day}^{-1}$), and gradually decreased thereafter. Unlike in apterae, in alatae the total volume decreased rapidly between day 0 and day 3 ($P < 0.01$,

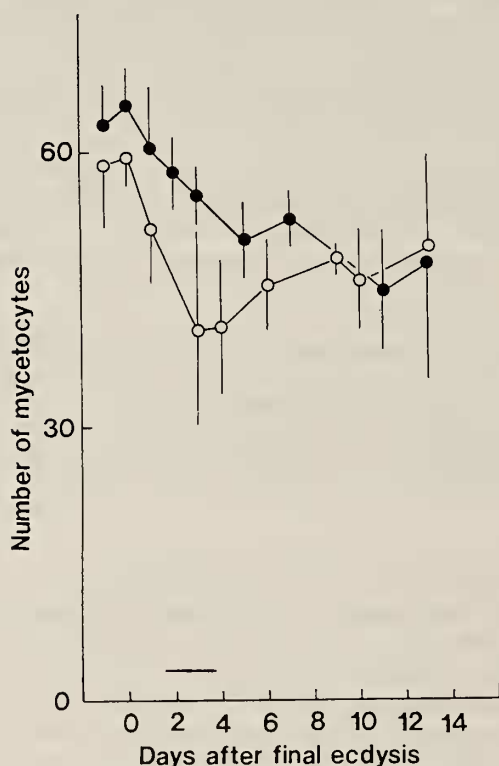


FIG. 1. Changes in number of mycetocyte per insect individual. Each value was expressed as mean $\pm$ SD. Closed symbols, apterae, $n=10$ to 25; open symbols, alatae, $n=10$ to 56. Horizontal bar designates the flying period.

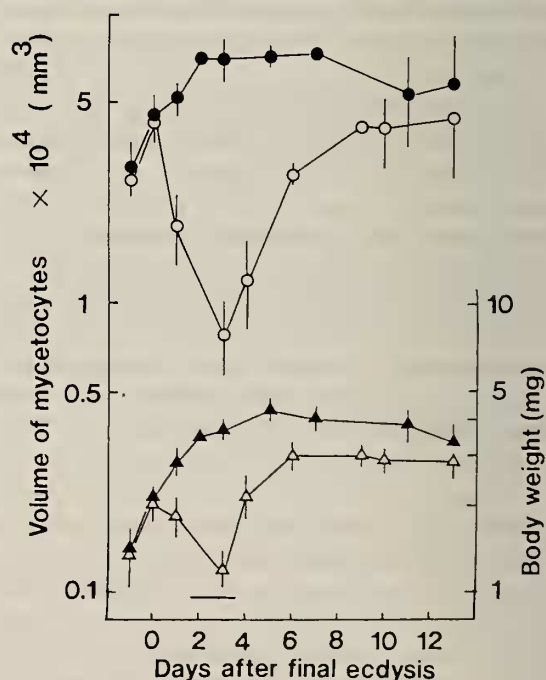


FIG. 2. Changes in median volume of mycetocytes (circles) and fresh body weight (triangles). Each value was expressed as mean $\pm$ SD. Closed symbols, apterae, $n=10$ to 25; open symbols, alatae, $n=10$ to 56. Horizontal bar designates the flying period.

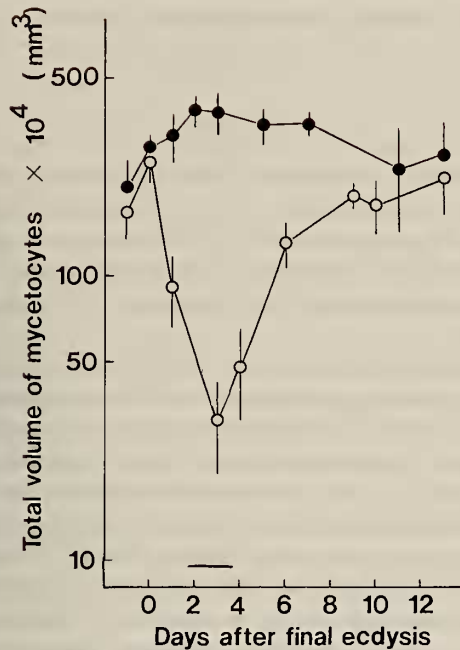


FIG. 3. Changes in total mycetocyte volume per insect individual. Total mycetocyte volume was calculated based on the number and mean diameter of mycetocytes for each individual. Each value was expressed as mean $\pm$ SD. Closed symbols, apterae; open symbols, alatae.

r.g.r.: $-0.70 \text{ mm}^3 \text{mm}^3 \text{day}^{-1}$, $-0.18 \text{ mgmg}^{-1} \text{day}^{-1}$), then increased between day 3 and day 9 ($P < 0.01$, r.g.r.: $0.30 \text{ mm}^3 \text{mm}^{-3} \text{day}^{-1}$, $0.16 \text{ mgmg}^{-1} \text{day}^{-1}$).

Density of endosymbiont

In the flying stage of alatae, when the size of mycetocytes marked the lowest value, the density of endosymbionts was also lowered. On re-settling, the density recovered 78% of the value on day 0 (Table 1).

TABLE 1. Variation in symbiont density with behavior in alatae

day (age)	behavior	n	density (* $10^5/\text{mm}^2$)
0 (4th instar)	settling	3	2.7 ± 0.2
3 (adult)	flying	8	1.5 ± 0.1
6 (adult)	settling	4	2.1 ± 0.1

Density was calculated by counting the total symbionts within several squares ($15 \times 15 \mu\text{m}$) on a $0.5 \mu\text{m}$ seciton around a median line of mycetocyte, and expressed as mean $\pm$ SD. 'n' Represents cell number surveyed. The values obtained were significantly ($P < 0.05$) different from one another.

Interestingly, the color of mycetocytes also changed at the flying stage in alatae. In apterae and larval stage of alatae, mycetocytes were usually colorless and transparent. As the mycetocytes in alatae became smaller in number and size after the final ecdysis, the cells took on the condensed green. On settling of the insect, the condensed green re-

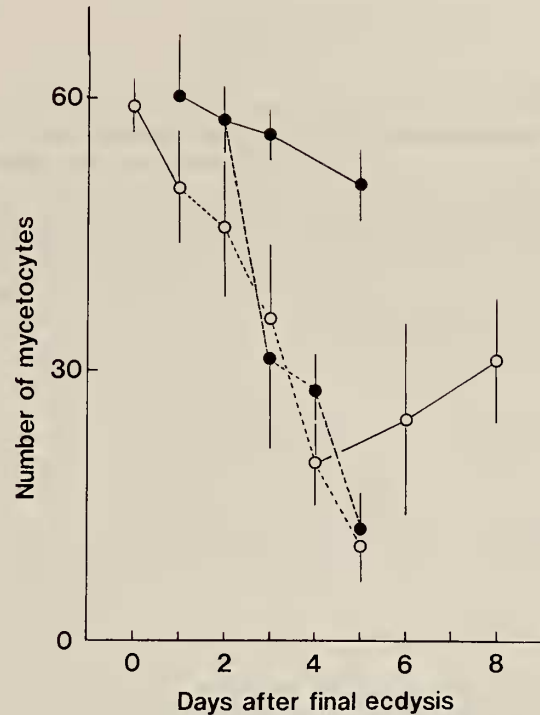


FIG. 4. Effects of starvation on number of mycetocyte per insect individual. Solid and dotted lines represent changes in fed and starved aphids, respectively. Each value was expressed as mean $\pm$ SD. Closed symbols, apterae, $n=5$ to 12; open symbols, alatae, $n=5$ to 10.

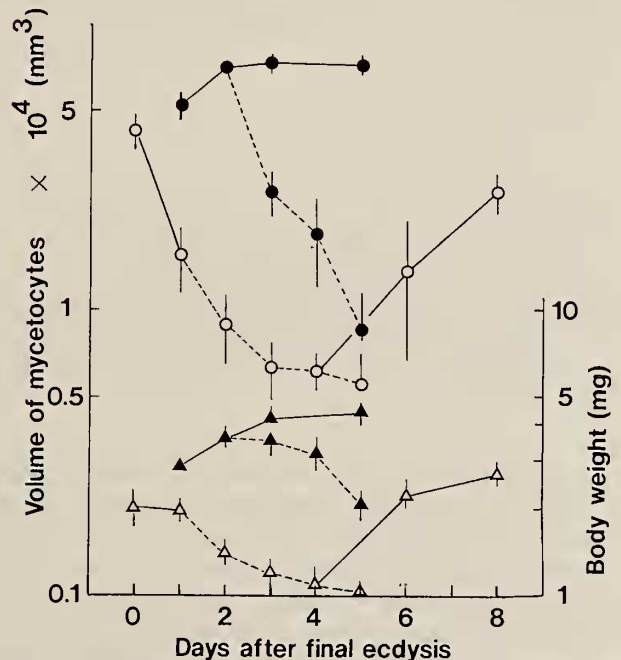


FIG. 5. Effects of starvation on volume of mycetocyte (circles), and fresh body weight (triangles). Solid and dotted lines represent changes in fed and starved aphids, respectively. Each value was expressed as mean $\pm$ SD. Closed symbols, apterae; open symbols, alatae.

mained only partially on mycetocytes, then faded away after all.

Effects of starvation on total volume of mycetocyte

Apteræ starved for 2 days did not much reduce their body weight (-12%), but the number and size of their mycetocytes declined by 45% and 75%, respectively (Figs. 4 and 5), and the total mycetocyte volume per aphid decreased rapidly down to 12% of the mean value on day 2 ($P < 0.01$, r.g.r.: $-1.2 \text{ mm}^3 \text{mm}^{-3} \text{day}^{-1}$, $0.18 \text{ mgmg}^{-1} \text{day}^{-1}$, for 3 days' starvation) (Fig. 6).

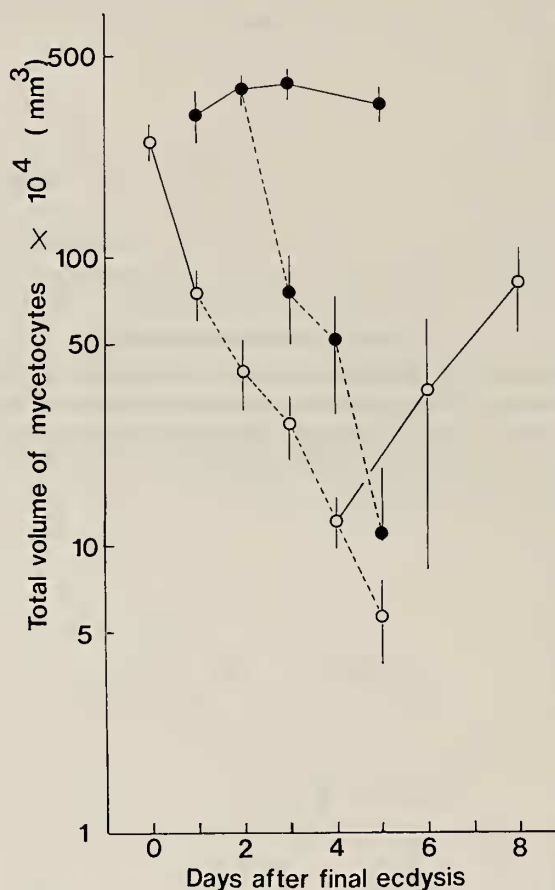


FIG. 6. Effects of starvation on total mycetocyte volume per insect individual. Solid and dotted lines represent changes in fed and starved aphids, respectively. Closed symbols, apteræ; open symbols, alatae.

Unlike apteræ, alatae decreased the number, volume and total volume of their mycetocyte spontaneously for the first one day after the final ecdysis even without starvation, and the starvation did not much reinforce the relative declining rate of the volume. In alatae, the decrease in the total mycetocyte volume was more gentle than in apteræ ($P < 0.01$, r.g.r.: $-0.60 \text{ mm}^3 \text{mm}^{-3} \text{day}^{-1}$, $0.20 \text{ mgmg}^{-1} \text{day}^{-1}$, for 3 days' starvation). When the starved alatae were transferred back to host plants, the total volume of mycetocytes and the aphid body weight began to increase rapidly, and 2 to 3 days later they started larviposition. The increase in number

of mycetocytes after refeeding was also significant ($P < 0.01$, between day 4 and day 7) (Fig. 4).

DISCUSSION

The present result on the changes in number and size of the mycetocyte in apteræ with age largely confirmed the results of the previous study [3]. In addition, our results on alatae provided several new findings with respect to the change of mycetocyte in response to their flight and feeding behavior.

Changes in number and size of the mycetocyte after the final ecdysis were quite different due to morph (Figs. 1 and 2). As a result, the change in the total volume of the mycetocyte was also remarkably different between the two morphs (Fig. 3). The most marked difference was represented by a steep decrease in size of the mycetocyte in alatae over 3 days after the final ecdysis (Fig. 2). This period exactly coincides with the period in which alatiform aphids develop their flight muscles to take off for migratory flight. Our previous study revealed that over the first two days after the final ecdysis alatae decrease their body weight, and the protein content of their indirect flight muscle increases in inverse proportion to their body weight [7].

In this period, alatae of *A. pisum*, like those of *Tuberolachnus salignus*, are not likely to ingest appreciable amount of food [7, 9], which suggests that their flight muscle goes on developing without supply of nutrients by feeding. And the fact that the sharp decrease of total mycetocyte volume had started when the whole body weight was not much reduced imply that this change is controlled selectively. In this context, the present results suggested that the putative source of nutrients for the developing muscle is the mycetocyte containing endosymbionts, since in alatae the rise and fall of the total volume of mycetocytes for the first several days after the final ecdysis was in exact inverse relation to that of the protein content of indirect flight muscle [7]. The result that not only the total volume of mycetocytes but also the density of endosymbionts in the mycetocyte decreased in this period (Table 1) may indicate that the endosymbionts rather than the mycetocyte itself serve as major nutrient sources.

The possibility that the mycetocyte harboring endosymbionts serve as a nutrient source to the host was also sustained by the experiments in which aphids were kept starved (Fig. 6). As far as aphids were kept starved, their total mycetocyte volume was consistently decreased at a much higher rate than that of the decline of their body weight, suggesting that under the nutritionally adverse conditions aphids will reduce their endosymbiotic system unfalteringly in order to preserve their own life at the cost of the endosymbionts. This situation has been most typically demonstrated with alatae at the teneral period. Starving them, on one hand, induced a sharp decline of their total mycetocyte volume (Fig. 6), and, on the other hand, prevented their indirect flight muscle from breaking down [7]. When the nutritional conditions were improved by refeeding, their total mycetocyte volume im-

mediately restored the original value, suggesting that the change is reversible in nature.

It had been rather unexpected that upon settling and refeeding of alatae their mycetocyte were tuned to increase in not only total volume (Fig. 3) but also cell number (Fig. 1). Refeeding after the starvation also resulted in increase in number of mycetocytes (Fig. 4). It is likely that under the nutritionally adverse conditions part of mycetocytes are decreased in size to the extent in which they are hardly distinguishable from fat body cells surrounding them, and that they restore the original size on refeeding. In any case, our conclusion that aphids survive the nutritionally adverse conditions at the cost of their endosymbionts will not be hurt. It is interesting to know what mechanisms make it feasible for the cells to change their size so extremely, and yet, reversibly.

Finally, it should be described that the present results concern only the maternal symbiosis. For the present, it is just a matter of conjecture whether or not under the adverse conditions aphids reduce the total symbiosis including that of their embryos. It would be very interesting to know under which circumstances, if at all, aphid embryos cooperate with their mother by reducing their own symbiosis.

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